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THE EFFECT OF HYPOPHYSECTOMY UPON THE RESPIRATORY QUOTIENT OF SNAKES¹

(One figure)

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THE influence of the pituitary on many physiological processes has been the object of ever increasing experimentation. Because of the diverse functions of the pituitary gland, research may follow any of several paths. In the study of the role of the hypophysis in carbohydrate metabolism, much has been done on the usual experimental animals—rabbit, rat, mouse, guinea pig, dog, and pigeon.

A résumé of the results of recent work leads to the conclusion that, in mammals, carbohydrate metabolism is probably altered by two pituitary factors. The first—a diabetogenic hormone—exerts a hyperglycemic action, tending to antagonize the effects of insulin on blood sugar. The second—a glycotropic factor—apparently exerts a restraining influence upon the oxidation of glucose and may also be concerned with gluconeogenesis. Young (1938) tested the action of anterior-lobe extracts and found that the diabetogenic action is obtained only after extended injections, whereas the glycotropic action is, by comparison, quite rapid. The former requires several days, while the latter is effective within a few hours.

Very little has been done on the hypophysis of the snake in any way. Schaeffer (1933) observed the effect of hypophysectomy and thyroidectomy in these animals, reporting that the former operation caused a decrease in size of the gonads, adrenals, and thyroid. Shedding of the skin after hypophysectomy was often more frequent and irregular, and thyroidectomy indicated by its similar results that the effect on shedding was due to decreased thyroid activity subsequent to hypophysectomy.

Extensive research on the normal respiratory physiology of large reptiles has been pursued by Benedict (1932), who observed oxygen consumption, respiratory quotient, relation between environmental and body temperatures, and other factors involved in normal respiration and metabolism.

SCOPE OF PRESENT WORK

The present work includes a series of R.Q. determinations on snakes before and after hypophysectomy. The former served to establish the normal R.Q. for the group of snakes and as self-controls, since all animals were subsequently hypophysectomized. After operation the second series was made, and the results of both series were compared statistically to determine if there was any significant difference between normal and

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operated R.Q. values. The effect of hypophysectomy on absolute oxygen consumption was determined simultaneously. Replacement therapy with mammalian pituitary extracts was attempted. High carbohydrate feeding to the operated group was tried, in an effort to study changes in ability to metabolize carbohydrate.

Snakes were chosen as experimental animals for several reasons.

1. With a normal feeding interval of 3-4 weeks, they are in a "basal" state for 2-3 weeks, so that a greater proportion of the time is available for testing.

2. Because of its extreme flexibility, the snake occupies a small space for a given body weight, thus reducing dead air space in the apparatus. A 900-gm. snake will readily occupy a space in which a 350-gm. rat is accommodated with difficulty.

3. Owing to the low activity level of snakes, it is much easier to obtain a record of oxygen consumption uncomplicated by excess activity.

4. Concomitant with low activity, as well as the expected lower metabolic rate in poikilothermic animals, the oxygen consumption is low, so the snake is preferable to smaller reptiles, such as lizards.

5. Hypophysectomy is a relatively simple operation because of the mandibular articulation, and the slight tissue trauma minimizes postoperative effects. There is also a high resistance to infection in the mouth.

6. The pituitary in reptiles lends itself beautifully to operation. The infundibular stalk is long and slender and directed backward from the forebrain. The *pars anterior* is only very loosely connected with it, if at all, and is actually posterior and ventral to the remainder of the gland. In the snakes there is no *pars tuberalis* (De Beer, 1926). With such loose attachment of the *pars anterior*, its separation can be accomplished with relative ease.

TECHNIQUES EMPLOYED

A. *General principles*.—The food of the snake is primarily protein, and an R.Q. somewhat lower than that of man might reasonably be expected after feeding. This quotient will soon decrease to fat level and remain there throughout the rather long fasting period. The maintenance of a normal blood sugar level soon depends upon conversion from non-carbohydrate sources, since the carbohydrate stores are depleted in a few days (Benedict, 1932). The glycerol of fat probably is one of the main sources of glucose, and the fat thus metabolized must exert a predominant influence upon the R.Q. level.

With the relatively small quantities involved in the respiratory activities of the snake, a closed-circuit method is more desirable for measuring them. The apparatus absorbs carbon dioxide in weighed soda lime tubes and graphically records the volume changes due to oxygen consumption. With these values the R.Q. can be calculated.

B. *Animal chamber*.—The snake, confined in a cylindrical wire-mesh basket having a hinged top and small side entrance covered by a sliding gate, is placed in the chamber proper—a copper container $4\frac{1}{2}$ inches high and 6 inches in diameter. The hinged cover of the latter closes into an oil-filled moat surrounding the rim of the chamber. Air enters through a tube in the bottom and passes out through a similar tube in the cover. A baffle under the cover diverts air to the periphery, assuring more complete circulation throughout the chamber. Air temperature of the chamber is determined by a thermometer (0.1° C. graduations) sealed in so that the outgoing air surrounds the bulb. The entire chamber is immersed in a water bath to within 1 inch of the top, with 6 liters of water providing close stabilization of internal temperature.

C. *Air impeller*.—Two cylindrical bells, $1\frac{1}{2}$ inches in diameter and 6 inches in length, operate in their respective oil-filled shells, which have central tubes projecting above the surface of the oil. The bells are suspended from a grooved wheel by a cord which raises and lowers them reciprocally. The wheel is given a reciprocating motion through an arm attached eccentrically to a larger rotating wheel, which is belt driven by a variable-speed motor. Hydrostatic displacement thus alternately draws in and expels air as the bells move up and down.

D. *Valves*.—The conventional rubber “flutter” valves allow the air to pass in only one direction. They are inclosed in housings having outlets and inlets so arranged that, in conjunction with the air-impeller unit, they maintain a unidirectional flow of air.

E. *Absorption tubes*.—The apparatus employs two sets, each consisting of two large glass tubes, the first filled with calcium chloride and the second filled half with soda lime and half with calcium chloride. One set is used to establish equilibrium in the system before the actual test, then the other is connected in place of the first, to determine gravimetrically the carbon dioxide absorbed during the test run.

F. *Spirometer*.—A light bell suspended in an oil-filled shell with a central tube comprises the spirometer. It is connected to the system so that uniform internal pressure is maintained, thus actually responding to volume changes. The bell size is such that 1 mm. of vertical movement is equivalent to 0.5 cc. The bell is delicately counterbalanced over a precision pulley by a silk thread attached to a counterweight. The latter bears an arm with an ink-writing pen, so that movements of the bell yield a continuous kymograph record of oxygen consumption. Contact of the pen with the paper is insured by twisting the thread to produce a moderate amount of torsion.

G. *Apparatus*.—The apparatus as a whole is shown in Figure 1. The air impeller, with the valves, is interposed between the absorption tubes and chamber and can be controlled to provide unidirectional air flow through the system at a rate of from 500 to 2,000 cc., or more, per minute. All joints are carefully sealed against leakage, either by winding with wire or rubber bands or by lanolin coatings. An inherent characteristic of the apparatus, tending to minimize leaks, is that with slow-moving, large-volume displacements actual pressure variations, equally positive and negative, are less than 0.5 cm. of water pressure. This places no strain on joints such as would be encountered with a conventional motor blower or pump.

ANIMALS USED

A. *General*.—The snakes used were of two species—*Pituophis sayi sayi* (Schlegel), the yellow bull snake, from Kansas; and *Elaphe guttata*, the corn snake, from Florida. Both species were generally tractable and amenable to handling. Frequent handling at times other than for actual testing conditioned them and minimized excitement due to similar manipulation previous to actual experimentation.

B. *Care*.—All snakes were kept in the same room, two to a cage, with winter-day temperature variations between 21° and 24° C. and never lower than 18° at night. In summer the variation was higher than this, but in all probability less than that encountered in the natural habitats. Apparatus temperature was approximated to that of the room, eliminating possible effects of altering the body temperature during testing.

Water was freely accessible at all times, and each animal was fed one or two mice every 3–4 weeks. They were disturbed as little as possible for 2 days after feeding, as excitement caused regurgitation of partly digested food by the more nervous snakes. They were all kept in good nutritional condition without the necessity of forced feeding.

Judging by the time of defecation of undigested detritus, as well as by the corroborative evidence of a return of the R.Q. toward fat level, the food was digested in 3-5 days. Benedict (1932), working with larger snakes, found that on a normal protein diet the caloric output returned to a basal level in a similar period. On the basis of the above-mentioned criteria, an interval of 6 or 7 days after feeding was considered ample to assure basal conditions for testing.

C. *Nutritional considerations.*—For a normal R.Q. of the fasting state the animal is assumed to be drawing its energy from fat stores. In mammals this condition is generally

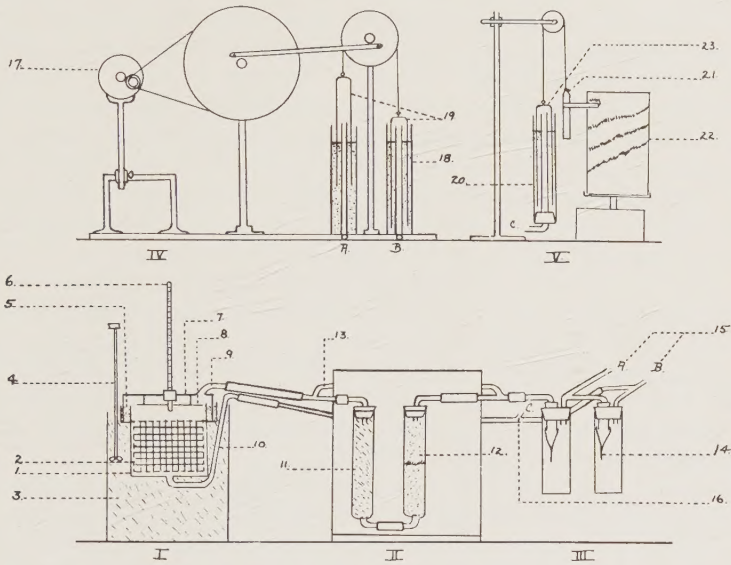


FIG. 1.—I, Animal chamber unit: 1, chamber proper; 2, wire basket for animal; 3, water bath; 4, water stirrer; 5, oil moat for lid; 6, thermometer; 7, lid; 8, baffle; 9, outlet tube; 10, inlet tube. II, Absorption tubes: 11, calcium chloride tube; 12, weighed tube, soda lime below, calcium chloride above; 13, lead to duplicate set of tubes (not shown). III, Valves: 14, rubber flutter valve; 15, leads to air impeller (connect to A and B on impeller); 16, lead to spirometer. IV, Air-impeller unit: 17, stirring motor, variable-speed cone drive; 18, shells filled with mineral oil; 19, reciprocating bells; A and B, leads connecting with valves. V, Spirometer unit: 20, shell filled with mineral oil; 21, counterbalance with ink-writing pen; 22, kymograph; 23, spirometer bell.

regarded as obtaining after food has been withheld for about 18-24 hours. After this time, the stores of glycogen are virtually depleted, and thereafter the animal subsists on its fat stores, as indicated by a lower R.Q.

Benedict (1932) observed that tortoises, on a diet high in carbohydrate, maintained an R.Q. level well above 0.7 for as much as 8 days after feeding. However, the principal food of snakes is protein; and for this reason it is quite unlikely that they have large glycogen storage, though there is no direct experimental evidence to refute or corroborate this conclusion. Its plausibility may be inferred from R.Q. results, which indicate a return to a prefeeding basal level in considerably less time than that required by the tortoise. In addition, fecal expulsion should preclude the possibility of further absorption of protein or its conversion into fat or carbohydrate from food sources. Therefore, any changes taking place are much more likely to be of a catabolic nature, with fat break-

down predominating. This will continue to predominate until all fat stores have been depleted—a condition not occurring in the present series, as shown by the presence of fat deposits on post mortem examination. The practice of feeding at 3–4-week intervals was deemed adequate, inasmuch as all animals showed a progressive weight and size increase.

In large snakes the effect of ingestion of protein has been studied by Benedict (1932). The so-called specific dynamic action of protein in mammals is well established and increases the metabolic rate from 30 to 40 per cent. A radically different situation exists in the snake. Shortly after food has been ingested, the heat output increases as much as 300 per cent. However, this is only temporary; and, in terms of total increment, Benedict found the cost of digestion of protein to be approximately 20 per cent, appreciably less than the cost for warm-blooded animals.

It can thus be seen that there is a nutritional economy in the digestion of a normal reptilian diet. Added to this is the general low level of metabolism common to poikilothermic animals. A fasted state of 3 or 4 weeks is a normal occurrence in the snake's existence and does not violate its normal physiological state in any manner which could be construed to introduce factors unnatural to the living habits of the animals used.

D. Testing techniques.—The snake to be used must have been fed no less than 5 days previously. The tube with the soda lime and calcium chloride is weighed to an accuracy of 1 mg. and connected into the circuit, but blocked so that air will pass through the duplicate set. The basket with the animal is placed in the chamber, the cover closed, and the air impeller started. Several minutes are allowed for attaining equilibrium in the system, while sufficient oxygen is added to fill the spirometer. At the start of the actual test the air circuit is shunted to the weighed tube, the start noted on the kymograph record, and the internal air temperature recorded.

The experiment may be continued as long as the spirometer capacity allows. Though this is only 60 cc., the oxygen consumption of the snake is so low that as much as 4 hours may elapse in using this amount. At the termination of the test the final chamber temperature is noted and the end indicated on the kymograph record. After the animal is removed from the chamber, the absorption tube is weighed and the barometric pressure recorded. If more than one test is desired, the air is shunted to the duplicate tubes, with the free leads blocked to keep the system closed. Without stopping the air impeller, oxygen is added and the system allowed to reach equilibrium. Meanwhile, the experimental tube can be weighed, reconnected to the system, and another test started within 5 minutes. By this method it is possible to obtain from three to six tests per day, since the snake is entirely passive and not affected by the confinement. Such a procedure also allows a check on possible diurnal variations, although these were found to be practically negligible.

In calculation, the volume of oxygen consumed is determined by measurement of the kymograph record and weight of carbon dioxide converted to volume at standard temperature and pressure. Oxygen volume is corrected to standard conditions, and a further correction is made if the temperature changed during the test. A change in temperature of 0.1°C . was found to be equivalent to 0.9 cc. of volume change, to be added or subtracted in accord with the direction of change in temperature.

NORMAL SERIES

Respiratory quotients.—The snakes were first used to establish a normal fasted R.Q. level and, with two exceptions, were tested several times to determine individual mean

respiratory quotients. The appearance of idiosyncrasies in the apparatus under operating conditions eliminated earlier results until all sources of error had been removed.

The testing extended from August, 1938, to May, 1940. Periodic advancement of individual animals from control group to operated group was made, as averages for each warranted the assumption that a satisfactory normal series had been obtained. In all, over one hundred individual determinations were made—a number deemed sufficiently large to average as a basis for the mean R.Q. Individual means ranged from 0.678 to 0.713, the mean of these being 0.699 (Table 1).

At first sight it might appear that some tests should be discarded because of their abnormally low values, but this apparent discrepancy was also encountered by Benedict (1932), who found some values at first attributed to technical error but later accepted, because, as experimentation progressed, it became evident that they occurred too frequently to be due to error. Such low values usually occurred when the environmental temperature was low, and at 18° C. he was able to maintain an R.Q. of 0.60 for several days in succession, with minimal values of 0.52. The present series of experiments did not embrace temperatures quite so low as this, and accordingly the minimal value, 0.56, also was above his lowest R.Q.

TABLE 1
NORMAL RESPIRATORY QUOTIENTS

Snake	Weight (Gm.)	No. of Tests	Maximum R.Q.	Minimum R.Q.	Mean R.Q.	Sigma of Mean	Snake	Weight (Gm.)	No. of Tests	Maximum R.Q.	Minimum R.Q.	Mean R.Q.	Sigma of Mean
<i>P1...</i>	415	13	0.760	0.630	0.709	0.011	<i>P9...</i>	310	10	0.736	0.632	0.700	0.011
<i>P3...</i>	390	7	0.820	0.560	0.698	0.065	<i>E1...</i>	535	7	0.797	0.641	0.702	0.019
<i>P4...</i>	470	5	0.727	0.654	0.704	0.010	<i>E2...</i>	310	19	0.810	0.600	0.703	0.014
<i>P5...</i>	135	6	0.750	0.564	0.687	0.028	<i>E3...</i>	210	8	0.715	0.691	0.701	0.003
<i>P6...</i>	450	5	0.760	0.600	0.700	0.025	<i>E4...</i>	175	6	0.729	0.667	0.693	0.008
<i>P8...</i>	350	7	0.715	0.664	0.698	0.007							

At present there is no adequate explanation for this phenomenon. The possibility of nitrogen's being given off was ruled out by Krogh (1906) and Oppenheimer (1906, 1907). Carbon dioxide retention was suspected, but Benedict calculated that retention sufficient to cause such R.Q. decreases would cause a weight change, and such was not detectable in his animals. Furthermore, the stored gas would eventually be released when the temperature rose, increasing the R.Q. above normal. This, too, did not occur.

The problem remains unanswered, and much remains to be learned about actual intermediate metabolic processes in reptiles; but the occurrence of low R.Q. values can be considered an actuality and not an artefact or technical error.

HYPOPHYSECTOMIZED SERIES

As mentioned earlier, the snake is admirably suitable for hypophysectomy. The *sella turcica* is a well-defined depression in the sphenoid bone and is readily located in the roof of the mouth. The jaw is opened at right angles to the body, and, after a midline incision through the oral mucosa, the bone is drilled with dental burrs. The anterior lobe is removed through the hole by suction with a fine glass pipette. Healing is rapid, with no incidence of infection. Intraperitoneal nembutal eliminates the difficulties of inhalation anesthetics incident to any oral surgery.

Postoperative conditions.—The operated animal is isolated for several days in a sterilized cage to avoid infection—which was never encountered—and then returned to its own cage. At least a week intervened between operation and testing.

Techniques on the hypophysectomized animals were the same as for normals. Oxygen consumption decreased, so that individual tests usually lasted longer; the fall in metabolic rate was approximately 25–40 per cent. As reported by Schaeffer (1933), the subsequent shedding shortly after operation served to check on the success of pituitary removal. Feeding was the same, and all animals accepted food readily. Since snakes are normally rather inactive, it would be difficult to determine if they were less active after operation. There was no apparent loss in speed or proficiency in catching mice.

One hundred and twenty-two tests on twelve animals showed mean R.Q. values ranging from 0.775 to 0.793 with a group mean of 0.778. The thirteenth snake showed no significant variation from the normal R.Q. and was found upon examination of histological sections of the pituitary region to have been incompletely hypophysectomized.

TABLE 2
HYPOPHYSECTOMIZED SERIES

Snake	No. of Tests	Maximum R.Q.	Minimum R.Q.	Mean R.Q.	Sigma of Mean	Snake	No. of Tests	Maximum R.Q.	Minimum R.Q.	Mean R.Q.	Sigma of Mean
<i>P1</i>	17	0.853	0.707	0.785	0.011	<i>P10</i>	10	0.797	0.770	0.780	0.003
<i>P4</i>	6	0.643	0.747	0.695	0.015	<i>P11</i>	10	0.788	0.770	0.778	0.003
<i>P5</i>	6	0.816	0.762	0.789	0.006	<i>E1</i>	13	0.849	0.748	0.793	0.008
<i>P6</i>	10	0.807	0.762	0.781	0.004	<i>E2</i>	6	0.797	0.745	0.775	0.007
<i>P7</i>	11	0.835	0.752	0.786	0.007	<i>E3</i>	7	0.806	0.767	0.779	0.006
<i>P8</i>	14	0.814	0.764	0.779	0.003	<i>E4</i>	10	0.826	0.757	0.782	0.007
<i>P9</i>	9	0.795	0.767	0.780	0.008						

When an experimental series on an animal was completed, it was killed, and the brain was removed, fixed, and sectioned, with particular care in the removal of all tissue from the *sella turcica*. Serial sections were examined for remnants of anterior-lobe tissue. With two exceptions, no significant amount was present. In *Elaphe* 2, there was a small proportion of scattered rests, and *Pituophis* 4, as previously cited, showed a considerable portion of active tissue. The former had the lowest mean R.Q. of the successfully operated series, and the latter had a normal R.Q. The correlation between R.Q. results and histological findings is evident (Table 2).

Replacement therapy.—Attempts were made, using mammalian pituitary extracts, to restore normal R.Q. levels. Although repeated massive doses were injected, no significant reduction in the R.Q. was obtained. This possibly is due to some interclass difference in pituitary hormones, so that mammalian hormones do not function properly to restore normal pituitary physiology in reptiles. That the operated results were due to trauma elsewhere in the brain is unlikely, because the peculiar anatomy of the snake pituitary minimizes such a possibility.

Carbohydrate feeding.—Feeding of killed mice, into which large amounts of concentrated carbohydrate solutions had been injected, was carried out in an attempt to study carbohydrate utilization by the snakes. Results were so variable that no conclusive evidence could be obtained.

DISCUSSION

Examination of the statistical data from the normal and operated series (Table 3) shows that the results are significant, with critical ratios more than three times the standard error of the difference.

As demonstrated by Houssay (1936), Schaeffer (1933), Fisher, Russell, and Cori (1936), and many others, hypophysectomy inevitably influences other endocrine glands. The thyroid is principally affected in causing a lower metabolic rate. Schaeffer reported a decrease in size of the thyroid by as much as one-third following hypophysectomy in snakes. However, this does not explain the altered R.Q., despite the decreased metabolic rate. The antagonism between pituitary hormones and insulin—a factor affecting carbohydrate metabolism—was shown by Houssay and Magenta (1927) to be independent of the thyroid.

TABLE 3
STATISTICAL SUMMARY OF RESPIRATORY QUOTIENTS

Snake	Mean Normal	Mean Operated	Difference	σ_m (Normal)	σ_m Operated	Sigma Difference	Critical Ratio
P1.....	0.709	0.785	+0.076	0.0109	0.0109	0.0154	4.9
P4.....	0.704	0.695	-0.009	0.0099	0.0153	0.0182	0.5
P5.....	0.687	0.789	+0.102	0.0266	0.0066	0.0274	3.7
P6.....	0.700	0.781	+0.081	0.0255	0.0045	0.0259	3.1
P7.....	0.700	0.786	+0.086	0.0196	0.0069	0.0209	4.1
P8.....	0.698	0.779	+0.081	0.0070	0.0034	0.0076	10.7
P9.....	0.700	0.780	+0.080	0.0106	0.0030	0.0114	7.0
P10.....	0.700*	0.780	+0.080	0.0196*	0.0027	0.0198	4.0
P11.....	0.700*	0.778	+0.078	0.0196*	0.0017	0.0194	4.0
E1.....	0.702	0.793	+0.091	0.0196	0.0081	0.0212	4.3
E2.....	0.703	0.775	+0.072	0.0140	0.0076	0.0159	4.5
E3.....	0.701	0.779	+0.078	0.0031	0.0060	0.0067	11.6
E4.....	0.693	0.782	+0.089	0.0083	0.0074	0.0106	8.4

* Average group mean, used in absence of individual normal series.

Houssay and associates found a factor in the pituitary complex having an action on blood sugar antagonistic to insulin and exerting a diabetogenic action in the depancreatized animal. Geiling *et al.* (1927), Young (1936), Corey and Britton (1937), Long and White (1938), and many others have definitely established the existence of this diabetogenic factor.

Fisher, Russell, and Cori (1936) showed that hypophysectomized rats oxidize glucose more rapidly than do normal rats, and this in turn accounts for the rapid decrease in blood sugar as well as in liver and muscle glycogen in the fasted hypophysectomized animals. This suggests that the pituitary tends to restrain carbohydrate oxidation and enables the maintenance of glycogen storage during the fast. Substitution therapy with anterior-lobe extract depresses the R.Q. to the normal fasting level and retards the disappearance of liver glycogen.

Russell and Bennett (1936) extended and confirmed these results; and Soskin *et al.* (1935), Weinstein (1939), Corey and Britton (1937), and Young (1936, 1938) used dogs similarly. Soskin approached the problem from the angle of glycogenesis. It was felt that the pituitary affected the formation of blood sugar rather than its usage. In the fasted animals there is no exogenous source of carbohydrate for glycogenesis, and so

glucose must be derived from protein and fat by glycconeogenesis. Hypophysectomized dogs showed failure of glycconeogenesis when, together with control animals, they were fed fat. The controls had a long survival rate, while the operated series survived no longer than if they had not been fed, indicating that they were unable to spare body protein by the utilization of ingested fat for glycconeogenesis.

Soskin, Levine, and Heller (1939) postulate the role of the pituitary by three possible routes—(a) directly, through the diabetogenic factor, (b) through a possible effect on the thyroid, which may alter protein-amino acid catabolism, thus secondarily influencing protein glycconeogenesis, and (c) a possible influence, still unexplained, on glycconeogenesis from fat.

Whether it is fully justifiable to apply these same theories to the snake is uncertain. This animal subsists almost entirely on fat and protein, except for the relatively small amounts of carbohydrate in the tissues of the animals eaten. Benedict (1932) states that snakes apparently have large fat storage, as indicated by a continued maintenance of a fat R.Q. for many weeks of fast.

In the present series, examination of internal organs revealed fat deposits in all cases, precluding the possibility of fat depletion causing protein breakdown after hypophysectomy. There was no apparent difference in the nature of these deposits in normal and operated animals, since they were mainly in the form of plaques in and around the mesenteries in both groups.

The experimental findings with the snakes would easily fit the theories advanced by Soskin, since the fat of the operated animals was apparently not adequately utilized and the higher R.Q. results could be attributed to the increased catabolism of protein for glycconeogenesis. If this were true, then the snake pituitary would seem to have functions similar to the mammalian pituitary, or at least possess a remarkable parallelism.

On the other hand, there might be some process of intermediate metabolism of fat or protein of a type as yet unknown in higher forms. Benedict stressed the lack of knowledge about the intermediate processes of metabolism in poikilothermic animals; and, until this has been studied in detail, the exact mechanism of the influence of hypophysectomy upon metabolism in snakes and its consequent reflection through the R.Q. will remain a theoretical assumption of similarity in the pituitary functions of snakes and mammals.

SUMMARY

1. One hundred and thirty R.Q. determinations on thirteen hypophysectomized snakes indicate that removal of the anterior lobe causes an increase in the R.Q. of fasted animals above that of fat metabolism. Such an increase is probably brought about by a decrease or loss of metabolic control by factors normally present in pituitary secretions.

2. Replacement therapy, attempted with mammalian pituitary extracts, gave no indication of return to a normal R.Q. This possibly was due to interclass variations in pituitary hormones.

3. Feeding experiments involving administration of carbohydrate were attempted, with results which could not be considered significant.

4. In order to carry out experimentation, apparatus was designed for studies on respiratory metabolism of small animals, in combination with an original type of air impeller, whose characteristics minimize pressure gradients and their attendant possibilities of leakage.

5. A simple approach to hypophysectomy was devised, eliminating many complicating factors normally present in the mammalian operation.

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